

Docket No. 648.46078X00

Serial No. 10/578,845

January 14, 2009**REMARKS**

Applicants have amended their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have cancelled claim 1, the sole independent claim being considered on the merits in the above-identified application, without prejudice or disclaimer, and have substituted therefor new claim 6. Claim 6 defines a method for fractionating polycyclic aromatic hydrocarbons using (a) a test solution in which a sample containing polycyclic aromatic hydrocarbons is dissolved in a solvent, (b) a plurality of eluents different from the solvent (in which the sample is dissolved) and different in polarity from each other, and (c) a column, the method including, inter alia, supplying a first eluent to the column and eluting contaminants in the test solution; mixing gradually a second eluent higher in polarity than the first eluent while reducing flow of the first eluent; and starting fractionating eluent solution when concentration of the second eluent with respect to the first eluent reaches a predetermined concentration, the polycyclic aromatic hydrocarbons being separated. Note, for example, pages 10 and 11 of Applicants' specification. Dependencies of claims 2 and 3 have been amended, in light of substitution of claim 6 for claim 1.

Initially, it is noted that the Examiner has indicated in the Office Action mailed October 14, 2008, that claims 1-4 are pending in the application; and the Examiner has rejected claims 1-3, claim 4 being withdrawn from consideration.

However, the Examiner's attention is respectfully directed to the Preliminary Amendment filed May 10, 2006, in which claim 5 was added to the application. Claim 5 expressly sets forth subject matter as in claim 3, but is dependent on claim 2, noting the amendment of claim 3 to delete multiple dependency in this Preliminary Amendment

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filed May 10, 2006. As the Preliminary Amendment was filed concurrently with the filing of the above-identified application, such Preliminary Amendment in its entirety must be entered. It is respectfully submitted that the Examiner has erroneously failed to consider claim 5. And it is respectfully submitted that upon further examination of the above-identified application, the Examiner must consider claim 5 as a claim first being considered in the above-identified application; and, if claim 5 rejected, the first Office Action rejecting claim 5 subsequent hereto cannot be made a Final rejection.

In any event, Applicants respectfully submit that all of the claims presented for consideration by the Examiner on the merits, that is, claims 2, 3, 5 and 6, patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims formerly in the application, that is, the teachings of U.S. Patent No. 5,076,909 to Overfield, et al., the Japanese patent documents to Matsushita, et al., No. 56-89058, and to Takamura, No. 6-94697, and the publication by Snyder, Introduction to Modern Liquid Chromatography (1979), pages 662-686, under the provisions of 35 USC 103.

It is respectfully submitted that the teachings of the references as applied by the Examiner would have neither disclosed nor would have suggested such a method for fractionating polycyclic aromatic hydrocarbons using (a) a test solution in which a sample containing polycyclic aromatic hydrocarbons is dissolved in a solvent, (b) a plurality of eluents to be a mobile phase for the test solution and different from the solvent in which the sample is dissolved and different in polarity from each other, and (c) a column packed with a packing material, and wherein the method includes, inter alia, supplying a first eluent to the column and eluting contaminants in the test solution; mixing gradually a second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent; and starting fractionating eluted solution when

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concentration of the second eluent with respect to the first eluent reaches a predetermined concentration, the polycyclic aromatic hydrocarbons being separated. See claim 6.

As will be discussed further infra, it is respectfully submitted that the combined teachings of the applied references would have neither disclosed nor would have suggested, in such a method for fractionating polycyclic aromatic hydrocarbons as in the present claims, and among other features therein, the gradual mixing of a second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent, with the fractionating of eluted solution being started when concentration of the second eluent with respect to the first eluent reaches a predetermined concentration; and, moreover, wherein the first eluent elutes contaminants in the test solution.

Furthermore, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such fractionating method as in the present claims, having features as discussed previously in connection with claim 6, and, additionally, wherein the solvent in which the sample containing polycyclic aromatic hydrocarbons is dissolved is an alcohol, one of the plurality of eluents includes dichloromethane, and an eluent lower in polarity than dichloromethane includes any one of n-hexane, carbon tetrachloride and toluene (see claim 2); and/or wherein the column is a silica gel column (see claims 3 and 5).

The present invention as claimed in the above-identified application is directed to a method for fractionating polycyclic aromatic hydrocarbons; this method can be used in connection with analysis of polycyclic aromatic hydrocarbons occurring in exhaust gas microparticulates contained in the atmosphere or in engine exhaust gas. The method claimed in the above-identified application can be utilized for fractionation of

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nitropolycyclic aromatic hydrocarbons, and, subsequently, quantitatively analyzing the nitropolycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, respectively.

Polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons contained in a solvent-soluble organosoluble fraction (SOF) in exhausts from diesel gas have been analyzed, although their contents are extremely small, because of their high carcinogenicity. Because the contents of these substances are small, these substances cannot be detected unless a large number of interfering substances are eliminated and a pretreatment performed, prior to analysis, to enhance the analytical sensitivity and precision. Due thereto, the sample to be tested is required to be subjected to a preanalysis treatment to eliminate interfering components in such a way that the sample adapts to the targets to be analyzed.

Various procedures for quantitative analysis of the polycyclic aromatic hydrocarbon have been proposed, as described on pages 3 and 4 of Applicants' specification; these proposed techniques include a third step of purification, which is a step of fractionating nitropolycyclic aromatic hydrocarbons or polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, by various techniques as described in the first full paragraph on page 4 of Applicants' specification.

However, the previously proposed techniques for the quantitative analysis have disadvantages, including the requirement of a large number of steps and a long operation time, wherein it is highly probable that fractionated substances are lost or decomposed; and there are also problems including a reproducibility problem, as described on pages 4-6 of Applicants' specification.

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Against this background, Applicants avoid disadvantages in previously proposed techniques, providing a method for fractionating polycyclic aromatic hydrocarbons, useful in the aforementioned purification step, which can efficiently and effectively separate the nitropolycyclic aromatic hydrocarbons, the polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, and the interfering substances from the SOF. Specifically, according to the present method, utilizing a column packed with a packing material and a plurality of eluents to be a mobile phase for the test solution, a first eluent is supplied to the column and contaminants in the test solution are eluted thereby; a second eluent which is higher in polarity than the first eluent is mixed gradually while reducing flow of the first eluent; and fractionation of the eluted solution is started when concentration of the second eluent with respect to the first eluent reaches a predetermined predetermined concentration. According to the present invention, interfering components can be eliminated from the target substance to be analyzed, in a relatively simple and effective manner, and an accurate analysis is achieved.

Matsushita, et al. discloses a chromatographic analytic method for hydrocarbon compounds that separates hydrocarbon compounds contained in a solution into three components, namely, saturates, unsaturates and aromatics, by means of high-performance liquid chromatography. The method includes use of an organochlorine solvent or a mixture of an organochlorine solvent and hydrocarbon solvent as an eluent; a first column that is filled with a silica-based filler; and a second column that is filled with a silver-containing silica-based filler, the two columns being connected in series, the first column serving to separate the hydrocarbon compounds into (saturates and unsaturates) and aromatics, and the saturates, unsaturates and aromatics in this order

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being eluted from the second column, without substantially affecting the separation of the components eluted from the first column, and these being introduced into a detector and detected quantitatively. Note the sole full paragraph on page 4 of the English translation of this patent document. See also the third full paragraph on page 5 and the first full paragraph on page 6, of this patent document.

It is respectfully submitted that Matsushita, et al. utilizes two columns, for providing the separation. It is respectfully submitted that this reference does not disclose, nor would have suggested, use of a plurality of eluents to be a mobile phase for the test solution, the plurality of eluents being different from the solvent in which the sample is dissolved and differing in polarity from each other, with a first eluent being supplied to the column and eluting contaminants, and a second eluent being mixed gradually which is higher in polarity than the first eluent while reducing flow of the first eluent, and with fractionation of the eluted solution being started when concentration of the second eluent with respect to the first eluent reaches a predetermined concentration, and advantages due thereto.

As can be seen in the foregoing with respect to Matsushita, et al., and also with respect to the teachings of Takamura, these references do not disclose, nor would have suggested, use of the plurality of eluents respectively to elute contaminants and the polycyclic aromatic hydrocarbons, with the recited gradual mixing of the second eluent higher in polarity than the first eluent while reducing flow of the first eluent, and with the start of fractionating eluted solution occurring when concentration of the second eluent relative to the first eluent reaches a predetermined concentration, as in the present claims.

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Takamura, et al. discloses an analytical method including passing a hydrocarbon oil to a first column and separating it into a saturated portion containing an olefin portion and an aromatic group portion by using a liquid chromatograph; applying a backflush to the first column after the saturated portion is eluted from the first column; passing the saturated portion eluted from the first column through a silver ion-carrying second column and separating it from the olefin portion; detecting the saturated portion from the silver ion-carrying second column by a differential refractometer and fractionating it; detecting the peak of the saturated portion and applying a backflush to the silver ion-carrying second column; detecting the olefin portion eluted from the silver ion-carrying second column by a differential refractometer and fractionating it; detecting the peak of the olefin portion and re-applying a backflush to the silver ion-carrying second column; and detecting the aromatic portion eluted from the silver ion-carrying second column by the differential refractometer and fractionating it. Note, for example, section [0016] on pages 10 and 11 of the English translation of Takamura.

As can be appreciated from the foregoing, Takamura has a relatively complex analysis procedure utilizing back-flushing and two columns, including a silver ion-carrying second column. It is respectfully submitted that the teachings of this reference do not disclose, nor would have suggested, the method as in the present claims, including steps discussed previously such as supplying the first eluent to the column and eluting contaminants; and mixing gradually the second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent, with fractionation of the eluted solution starting when concentration of the second eluent with respect to the first eluent reaches a predetermined concentration, and advantages thereof.

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It is respectfully submitted that the additional teachings of Snyder and of Overfield, et al. would not have rectified the deficiencies of either of Matsushita, et al. or of Takamura, such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Snyder, as applied by the Examiner, describes gradient elution, disclosing that gradient elution devices provide for the admixture of two (or more) solvents, a weak initial solvent A and a strong solvent B, so that the concentration of B in the mobile phase entering the column increases throughout the separation; and, as a result, the mobile phase provides large K' values for the sample of interest at the beginning of separation and small K' values at the end of separation, that is, the solvent is initially weak and becomes progressively stronger as separation proceeds. See page 664 of Snyder.

The Examiner contends that Snyder "discloses increasing polarity of the eluents enhances detection sensitivity, improves resolution and decreases column deterioration", and also contends that this article on page 684 "discloses that hexane and dichloromethane are an eluting pair to increase polarity for liquid solid liquid chromatography". The Examiner is respectfully requested to point out the specific portion of Snyder that discloses increasing polarity of the eluents enhances detection sensitivity, improves resolution and decreases column deterioration.

Overfield, et al. discloses a method for the spectroscopic analysis of solutions containing at least two aromatic compounds for the aromatic core content, including steps of irradiating the solution with UV light having a wavelength range of which at least a portion is within the range of about 200 nm-500 nm; measuring absorbance; deriving the integral of absorbance as a function of photon energy; and comparing the

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absorbance integral to a predetermined value, to thereby obtain the aromatic core content. As applied by the Examiner, note from column 19, line 18, through column 20, line 59, of this patent. This disclosure includes a description that, initially, a weak solvent is employed which is substantially transparent to UV radiation and has a sufficient solubility parameter to dissolve all components of the sample to be introduced into the column but of which the solubility parameter is not so high that relatively sharp discrimination between different chemical types in the sample by high performance liquid chromatography will not be possible; during a first period of operation a sample of the hydrocarbon which is to be analyzed is passed to a location where it co-mingles with a weak solvent to form a substantially uniform solution which is free of precipitated materials such as asphaltenes; and, subsequently, when substantially all the saturated and aromatic hydrocarbon molecules have been eluted from the column by the weak solvent, as evidenced by a decline in the UV absorption and refractive index to virtually their base values with the solvent only, a strong solvent (that is, a solvent having relatively high polarity) is passed into the column by at a progressively increasing rate while the weak solvent is pumped at a correspondingly progressively reducing rate so that the total volume-rate of solvent is substantially unaltered, and after a selected third time period the weak solvent is totally absent and the only solvent passing to the upstream end of the column is the strong solvent.

Even assuming, arguendo, that the teachings of Snyder and of Overfield, et al. as applied by the Examiner were properly combinable with the teachings of either of Matsushita, et al. or of Takamura, the combined teachings would have neither disclosed nor would have suggested such features of the present invention as discussed previously, including the supply of first eluent to elute contaminants and mixing

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gradually the second eluent higher in polarity than the first eluent while reducing flow of the first eluent, with fractionation of the eluted solution starting when concentration of the second eluent with respect to the first eluent reaches a predetermined concentration, as in claim 6; and/or additional features of the present invention as in the other claims being considered on the merits herein.

Contentions by the Examiner in the paragraph bridging pages 2 and 3 of the Office Action mailed October 14, 2008, with respect to differences between each of the primary references (Matsushita, et al. and Takamura) and the previously considered claims, are noted. However, it is respectfully submitted that the presently claimed invention differs from the teachings of these references by much more than the recitation of increasing the polarity of the eluents; and it is respectfully submitted that the teachings of the primary references, even in combination with the teachings of the secondary references as applied by the Examiner, would have neither disclosed nor would have suggested supply of the first eluent to elute contaminants, with the mixing of the second eluent and the starting of the fractionation, as in the present claims, and advantages thereof.

In view of the foregoing comments and amendments, reconsideration and allowance of all claims presently pending in the above-identified application are respectfully requested.

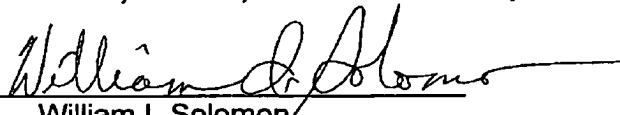
To the extent necessary, Applicants hereby petition for an extension of time under 37 CFR 1.136. Kindly charge any shortage of fees due in connection with the

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Respectfully submitted,

ANTONELLI, TERRY, STOUT & KRAUS, LLP

By 
William I. Solomon
Registration No. 28,565

WIS/ksh
1300 N. 17th Street, Suite 1800
Arlington, Virginia 22209
Tel: 703-312-6600
Fax: 703-312-6666